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# LEVEL AND METHOD OF SUPPLEMENTATION OF VITAMIN C ON PERFORMANCE AND THERMOREGULATORY RESPONSES OF RABBITS RAISED IN A HUMID TROPICAL ENVIRONMENT

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## ABSTRACT

A total of 40 crossbred weaner rabbits, aged 4-6 weeks, weighing on average 450 g were bought from a Rabbitry and allowed to adjust for one week. The rabbits were randomly allocated in a 4x2 factorial arrangement made up of four vitamin C treatments (100, 200, 300 and 400 mg) and two methods of supplementation (in feed or water) in a completely randomized design. Results obtained showed that body weight and feed intake were significantly ($p<0.01$) higher for rabbits on 200 mg vitamin C, however average daily gain and feed to gain ratio were not significantly ($p>0.05$) different for all vitamin C levels. Rabbits on 200 and 300 mg vitamin C had significantly ($p<0.01$) higher water intake than 100 and 400 mg. Method of supplementation did not affect ($p>0.05$) feed intake, average daily gain and feed to gain ratio. Body weight and water intake were significantly ($p<0.01$) affected by supplementation method being higher in water than in feed supplemented rabbits. Daily weight gain, feed to gain ratio and weight gain did not show any significant ($p>0.05$) interaction between vitamin C level and method of supplementation however, there was interaction ($p<0.05$) on feed intake, water intake and body weight. There was significant ($p<0.05$) interaction between supplementation level and method on respiratory rate of rabbits in the morning but not in the afternoon. Skin temperature was higher in rabbits supplemented with 100 and 200 mg vitamin C in feed than in water the reverse being the case at 300 and 400 mg vitamin C in the morning while in the afternoon skin temperature was higher in rabbits supplemented in feed at 100, 300 and 400 mg vitamin C than in water. Rabbits supplemented with 100 and 200 mg vitamin C had higher ear temperature in the morning, while in the afternoon, rabbits on 100 and 300 mg vitamin C in feed had higher ear temperature than in water. Afternoon rectal temperature showed significant ($p<0.05$) interaction between vitamin C level and method of supplementation. Rectal temperature was lower in rabbits supplemented with 100 and 200 mg vitamin C in water in the morning and 100, 200 (water) and 300 mg vitamin C (feed) in the afternoon. Based on the results, 200 mg vitamin C in water and 300 mg vitamin C in feed is recommended to ease heat stress effect on rabbits.

**Keywords:** Vitamin C, Feed, water, thermoregulatory, performance, rabbits

## INTRODUCTION

The breeding of rabbits is expanding; this is mainly attributable to the rabbit's high rate of reproduction, high genetic selection potential, rapid growth rate, early maturity, efficient feed utilization, high quality nutritious meat, and limited competition with humans for similar foods (Habeeb *et al*., 1996; Ayyat and Marai, 1997). However, the most obvious limitation to

rabbit production in regions with hot climate is the susceptibility of this species to heat stress (Ondruska *et al.,* 2011). Susceptibility to heat stress produces a series of changes in biological functions which in turn lead to impairment of production and reproductive function (Marai *et al.*, 2007) as is observed in the hot tropics especially areas close to the equator. Such detrimental effects are more evident during the hot season of the year when ambient temperatures can be as high as 30-40°C. The effect of heat stress is aggravated when heat is accompanied by high humidity (Habeeb *et al.*, 1993; Marai *et al.,* 2001). The changes that occur in the rabbit's biological functions are depressed feed intake, feed efficiency, utilization, and disturbance in metabolism of water, protein, energy, mineral balance, enzymatic reaction, hormonal secretions and blood metabolites (Marai *et al.*, 2002).

In rabbits, stress associated with exposure to high ambient temperature decreases growth performance, possibly because it destroys cellular biological molecules (Liu *et al.,* 2011). Rabbits, being homoeothermic, can regulate the heat input and output of their bodies using physical, morphological, biochemical, and behavioural processes to maintain a constant body temperature (Marai and Habeeb, 1994). During stress episode, reactive oxygen species (ROS) generation exceeds the body antioxidant production capacity, and oxidative stress develops (Roth, 2000). Vitamins are organic substances without energetic value, but necessary for the metabolism of animals but are not synthesized in adequate quantities hence vitamins must be provided by feeds or through the intestinal flora activity (Lebas, 2000). They act at very small concentrations mainly as co-enzymes or precursors, but are never incorporated as constitutive part of the body. Vitamin C is regularly used to alleviate heat stress in rabbits. This study was conducted to evaluate the effect of level and method of supplementation of vitamin C on performance and thermoregulatory response of growing rabbits raised in a humid tropical environment.

## MATERIALS AND METHODS

### Experimental Site

The experiment was carried out in the Rabbitry Unit of the Livestock Teaching and Research farm, University of Agriculture, Makurdi. Benue state, Makurdi, is located on latitude 17°14'' North and Longitude 8°31'' East, 90m above sea level. Makurdi has a tropical climate with distinct wet and dry season. The mean temperature of the research site ranged from 22-23°C in the morning and 26-30°C in the afternoon during the study period in August to September. The mean relative humidity ranged from 80-88% in the morning and 78-85% in the afternoon. The area receives a well distributed rainfall of 1108mm during the rainy season (April – October) with a peak in July/August. The dry season has little or no rainfall.

### Experimental Animals and Management

A total of 40 cross bred weaner rabbits of both sexes were bought from a Rabbitry within Makurdi metropolis. The rabbits were 4 – 6 weeks of age and weighed between 400 to 500 g. The rabbits were randomly allocated to four vitamin C supplementation levels (100, 200, 300 and 400 mg) and two supplementation methods (in feed or water) in a 4x2 factorial arrangement in a completely randomized design. The rabbits were kept in wooden and wire mesh cages that were raised 62 cm above the ground. The cages were kept in a well-ventilated shed completely fenced with wire mesh. The feeders were made from cut beverage tins fastened to the cages with wire to avoid wastage of feed. Drinkers were made from portable plastic containers firmly tied to the cages. All sanitary management measures were properly carried out during the period of experimentation. Before the commencement of the experiment, animals were treated against *endo* and *ecto* parasites with ivomectin (0.2 ml/kg body weight).

Feed grade vitamin C was obtained from Natural remedies, herbal feed supplement. The usage of which is to optimize the body resistance thereby livability. Vitamin C was included in the 18%CP and 2500 ME/kg DM concentrate diet containing maize offal 63.10, soybean meal (full fat) 33.40, bone meal 3.0, salt 0.25 and vitamin/mineral premix 0.25 at varying levels of 100, 200, 300, and 400 mg/kg feed for the first group, while the concentrate diet was offered to the second group supplied with vitamin C at 100, 200, 300 and 400 mg in 250 ml drinking water. Feed and water were provided ad-libitum. The experiment was conducted in the rainy season and lasted for 8 weeks, within the period of August to September, 2015.

Performance records taken were feed intake, water intake, and weight changes. 50g of feed was weighed and offered to the rabbits daily in the morning at 8 am at the start of the study and was gradually increased to 100g per day as they grew bigger and older. The leftover feed was weighed the next day before feeding. The rabbits were given 300 ml of water daily and the leftover water was measured using a calibrated cylinder the following day. Weight change was monitored using 20 kg calibrated scale, to weigh the rabbits weekly.

Collection of thermoregulatory data was done weekly, in the morning at 8 am and afternoon at 3 pm, to determine ear, skin and rectal temperatures using a professional instant digital thermometer with temperature range of 32 – 45$^{o}$C and temperature reading within 10 – 15 seconds, while respiratory rate and heart rate were measured using a stop watch and a stethoscope. The skin temperature was measured by placing the sensitive point of the digital thermometer on the neck region of the rabbit. The ear temperature was measured by placing the sensitive point of the digital thermometer in the outer ear of the rabbit. Rectal temperature was measured by inserting the sensitive unit of the digital thermometer in the rectum at 1cm depth. Respiratory rate was measured while the rabbit was at rest by visually counting the flank movement for one minute using a stop watch. Heart rate was measured using a stethoscope which was placed on the left region of the thoracic vertebrae of the rabbit and the heart rhythms were counted for one minute timed with a stop watch. The ambient temperature and relative humidity of the Rabbitry was measured using a digital temperature-humidity clock at 8 am and 3 pm daily.

**Statistical Analysis**

Data obtain from the study were analyzed using the generalized linear model procedure of SAS for one-way analysis of variance. Means were separated using pair-wise difference (SAS, 2002).

## RESULTS

Performance of growing rabbits to vitamin C supplementation is shown in Table 1. Body weight and feed intake were significantly ($p<0.01$) higher for rabbits on 200 mg vitamin C than the other levels though average daily weight gain and feed to gain ratio were not significantly ($p>0.05$) different for all the vitamin C levels. Rabbits on 200 and 300 mg vitamin C had significantly ($p<0.01$) higher water intake than those on 100 and 400 mg. Method of vitamin C supplementation did not affect ($p>0.05$) feed intake, average daily weight gain and feed to gain ratio (Table 2). Body weight and water intake were however significantly ($p<0.01$) affected by supplementation method being higher in rabbits supplemented with vitamin C in water than in feed.

**Table 1: Effect of vitamin C level on performance of weaner rabbits raised in a humid tropical environment**

| | Vitamin C level (mg) | | | | |
|---|---|---|---|---|---|
| Parameter | 100 | 200 | 300 | 400 | P-value |
| Body Weight (kg) | 1.05±0.26[b] | 1.79±0.26[a] | 1.09±0.27[b] | 1.08±0.23[b] | 0.0067 |
| Feed intake (g/day) | 34.97±1.70[b] | 45.09±1.70[a] | 36.75±1.78[b] | 35.20±1.49[b] | <.0001 |
| Water intake (ml/day) | 169.59±8.56[b] | 206.25±8.57[a] | 209.20±8.44[a] | 159.86±7.51[b] | <.0001 |
| Average daily gain (g) | 12.74±2.38 | 15.39±2.36 | 15.51±2.49 | 13.10±2.09 | 0.7356 |
| FGR (feed/gain) | 1.84±0.30 | 2.53±0.30 | 1.78±0.32 | 2.07±0.27 | 0.2909 |

[ab]Means with different superscripts along rows are significantly different (p<0.05). FGR- Feed to gain ratio.

**Table 2: Effect method of vitamin C supplementation on performance of weaner rabbits raised in a humid tropical environment**

| | Method of supplementation | | |
|---|---|---|---|
| Parameter | Feed | Water | P-value |
| Body Weight (kg) | 1.01±0.19[b] | 1.20±0.17[a] | <.0001 |
| Feed intake (g/day) | 37.40±1.23 | 38.61±1.13 | 0.4696 |
| Water intake (ml/day) | 154.98±6.19[b] | 217.48±5.70[a] | <.0001 |
| Average daily gain (g/day) | 12.56±1.71 | 15.81±1.59 | 0.1643 |
| FGR (feed/gain) | 2.22±0.22 | 1.88±0.20 | 0.2458 |

[ab]Means with different superscripts along rows are significantly different (p<0.05). FGR Feed to gain ratio.

The interaction between vitamin C level and method of supplementation on performance of weaner rabbits is shown in Table 3. Daily weight gain, feed to gain ratio and weight gain did not show any significant interaction between vitamin C level and method of supplementation however, there was interaction (p<0.05) between vitamin C level and method of supplementation on feed intake, water intake and body weight. Feed intake was similar for feed and water supplemented vitamin C on 100, 300 and 400 mg vitamin C while on 200 mg vitamin C, it was higher in water than feed supplemented group. Water intake was similar for feed and water supplemented 100 mg vitamin C rabbits while it was higher in water supplemented than feed supplemented rabbits on 200, 300 and 400 mg vitamin C. For body weight, 100 and 400 mg vitamin C in feed and water had similar weight while it was higher in water than feed supplemented vitamin C on 200 and 300 mg vitamin C. Daily weight gain was also higher in rabbits supplemented with 200 and 300 mg vitamin C in water than feed.

Table 4 shows the effect of vitamin C on thermoregulatory response of growing rabbits. There was a no significant difference in morning heart rate, afternoon heart rate, morning respiratory rate, afternoon respiratory rate, morning ear temperature, afternoon ear temperature, morning skin temperature, afternoon skin temperature, morning rectal temperature, afternoon rectal temperature. In Table 5 there was no significant difference in

morning heart rate, afternoon heart rate, morning respiratory rate, afternoon respiratory rate, morning ear temperature, afternoon ear temperature, morning skin temperature, afternoon skin temperature, morning rectal temperature, afternoon rectal temperature.

**Table 3: Effect of Level of Vitamin C and Method of Supplementation on Performance of Weaner Rabbits Raised in a Humid Tropical Environment**

| Level | 100 | | 200 | | 300 | | 400 | | |
|---|---|---|---|---|---|---|---|---|---|
| Method | Feed | Water | Feed | Water | Feed | Water | Feed | Water | P-Value |
| BW (kg) | 0.97±0.41$^{c}$ | 1.13±0.32$^{bd}$ | 1.05±0.38$^{c}$ | 1.36±0.35$^{a}$ | 0.95±0.38$^{c}$ | 1.23±0.38$^{b}$ | 1.08±0.32$^{cd}$ | 1.07±0.32$^{cd}$ | 0.003 |
| DFint (g/day) | 36.40±2.69$^{bc}$ | 33.54±2.00$^{c}$ | 42.86±2.49$^{b}$ | 47.32±2.33$^{a}$ | 33.93±2.49$^{bc}$ | 39.58±2.53$^{b}$ | 36.41±2.14$^{bc}$ | 34.00±2.08$^{c}$ | 0.054 |
| DWint (g/day) | 150.27±13.52$^{c}$ | 188.91±10.48$^{b}$ | 161.35±12.52$^{bc}$ | 251.17±11.71$^{a}$ | 169.57±12.52$^{bc}$ | 248.84±12.75$^{a}$ | 138.73±10.75$^{c}$ | 181.00±10.48$^{b}$ | 0.070 |
| ADG (g/day) | 12.80±3.77 | 12.68±2.92 | 11.58±3.42 | 19.20±3.26 | 12.50±3.49 | 18.52±3.55 | 13.35±2.99 | 12.86±2.92 | 0.486 |
| FGR | 2.07±0.48 | 1.61±0.37 | 2.53±0.43 | 2.52±0.41 | 1.84±0.44 | 2.45±0.38 | 2.45±0.38 | 1.68±0.37 | 0.778 |

$^{ab}$Means with different superscripts along rows are significantly different ($p<0.05$). BW=Body Weight, DFint= Feed intake, DWint= Water intake, ADG= Average daily gain, FGR= Feed to gain ratio.

**Table 4: Effect of Vitamin C level on Thermoregulatory Response in weaner rabbits raised in a humid tropical environment**

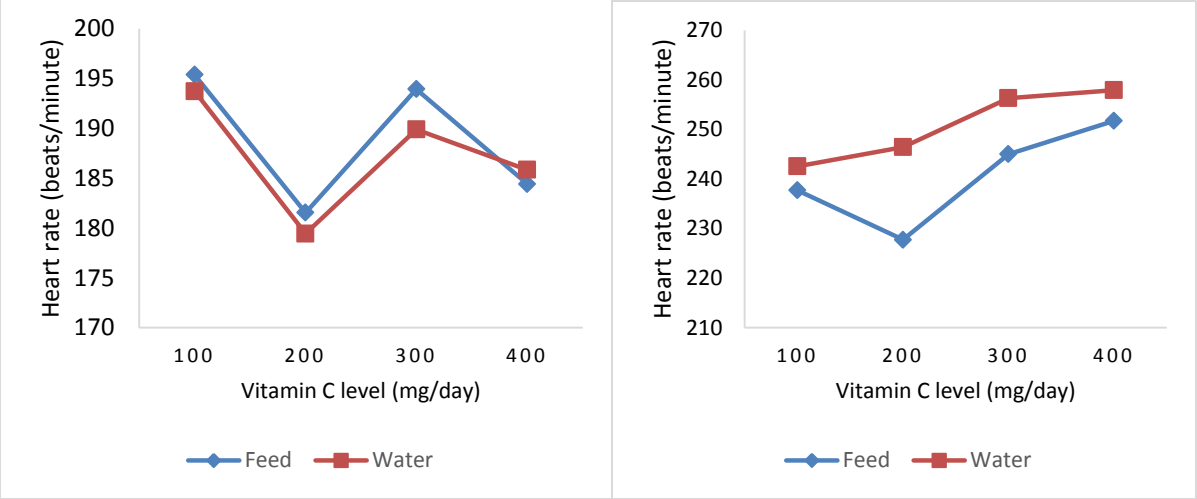
| Parameter | Circadian rhythm | Vitamin C level (mg) | | | | P-value |
|---|---|---|---|---|---|---|
| | | 100 | 200 | 300 | 400 | |
| Heart rate (bpm) | Morning | 194.55±4.57 | 180.49±4.53 | 191.91±4.77 | 185.14±4.01 | 0.139 |
| | Afternoon | 240.14±15.23 | 237.09±15.35 | 250.65±16.10 | 254.82±13.39 | 0.801 |
| Respiratory Rate (cpm) | Morning | 125.55±4.87 | 125.66±4.88 | 129.28±5.09 | 135.65±4.27 | 0.343 |
| | Afternoon | 131.26±6.36 | 142.51±6.41 | 132.67±6.72 | 142.69±5.59 | 0.402 |
| Ear temperature (°C) | Morning | 34.32±0.15$^{a}$ | 34.92±0.15$^{ab}$ | 35.31±0.16$^{b}$ | 35.22±0.13$^{b}$ | 0.052 |
| | Afternoon | 35.93±0.18 | 35.91±0.19 | 36.16±0.19 | 35.75±0.16 | 0.502 |
| Skin temperature (°C) | Morning | 37.30±0.16 | 37.15±0.16 | 37.62±0.16 | 37.41±0.14 | 0.211 |
| | Afternoon | 37.33±0.18 | 36.97±0.18 | 37.58±0.91 | 37.24±0.16 | 0.147 |
| Rectal temperature (°C) | Morning | 38.18±0.13 | 38.07±0.13 | 38.11±0.13 | 38.07±0.11 | 0.917 |
| | Afternoon | 38.28±0.11 | 38.27±0.11 | 38.35±0.11 | 38.31±0.09 | 0.968 |

$^{ab}$Means with different superscripts along rows are significantly different ($p<0.05$)

**Table 5: Effect of vitamin c supplementations methods on thermoregulatory response in weaner rabbits**

| Parameter | Circadian rhythm | Method of supplementation | | P-value |
|---|---|---|---|---|
| | | Feed | Water | |
| Heart rate (bpm) | Morning | 188.82±3.29 | 187.22±3.04 | 0.676 |
| | Afternoon | 240.55±11.08 | 250.80±10.19 | 0.496 |
| Respiratory rate (cpm) | Morning | 128.74±3.52 | 129.32±3.24 | 0.903 |
| | Afternoon | 133.83±4.63 | 140.74±4.25 | 0.273 |
| Ear temperature (°C) | Morning | 35.02±0.11 | 35.11±0.10 | 0.544 |
| | Afternoon | 36.08±0.13 | 35.79±0.12 | 0.404 |
| Skin temperature (°C) | Morning | 37.40±0.11 | 37.34±0.10 | 0.702 |
| | Afternoon | 37.39±0.13 | 37.17±0.12 | 0.234 |
| Rectal temperature (°C) | Morning | 38.21±0.09 | 38.01±0.08 | 0.110 |
| | Afternoon | 38.30±0.08 | 38.30±0.07 | 0.989 |

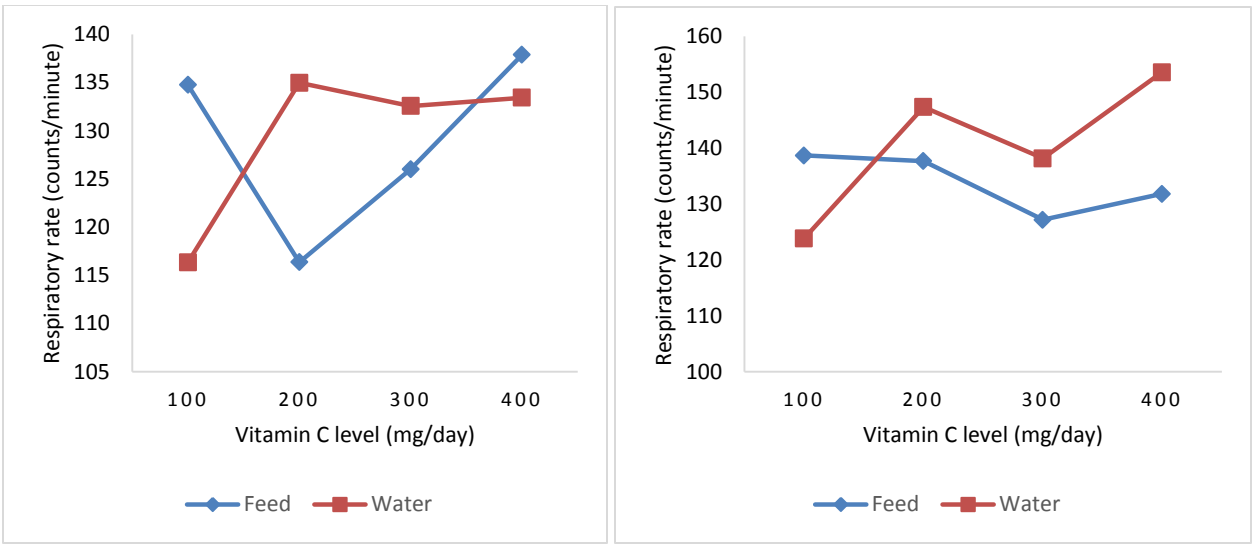
Heart rate of the rabbits (Fig. 1) in the morning was slightly higher in feed than water supplemented groups being lower on 200 and 400 mg vitamin C supplementation in feed and water. In the afternoon however, rabbits supplemented with vitamin C in water had higher heart beat than those supplemented in feed at all levels of supplementation.




Morning Afternoon

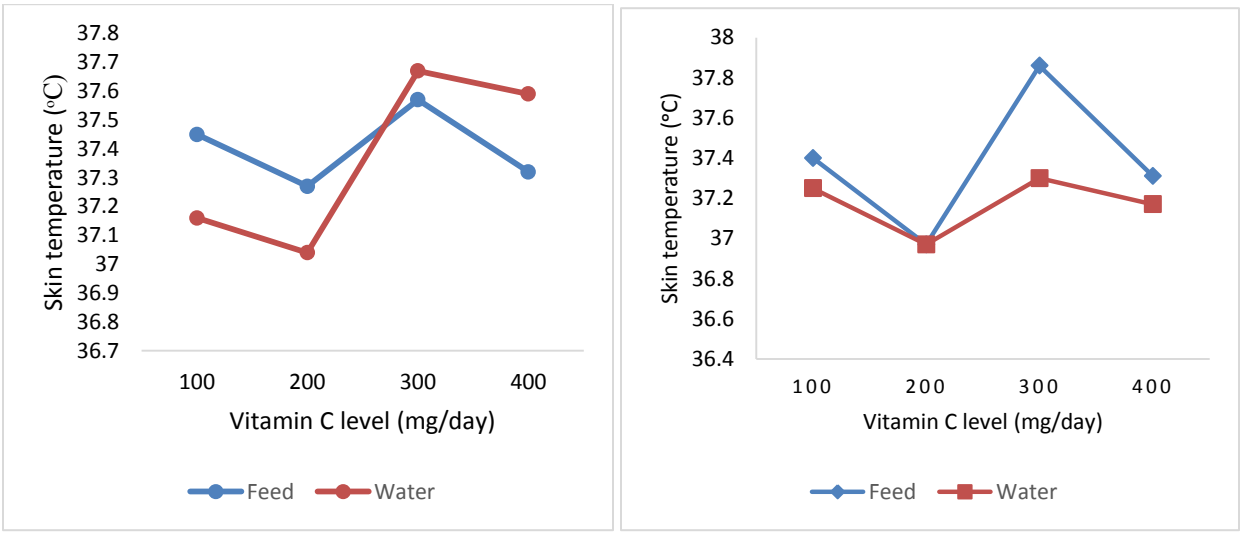
**Figure 1:** Heart rate of growing rabbits supplemented with vitamin C in feed and water in a humid tropical environment.

There was significant ($P<0.05$) interaction between supplementation level and method on respiratory rate of rabbits in the morning but not in the afternoon (Fig. 2). In the morning, at 100 and 400 mg vitamin C, respiratory rate was lower in water than feed supplemented groups, however on 200 and 300 mg vitamin C, it was higher in water supplemented than feed supplemented groups. In the afternoon, respiratory rate was higher in feed than water supplemented rabbits on 100 mg vitamin C while the reverse was the case at other levels of vitamin C supplementation. Skin temperature (Fig. 3) of growing rabbits was higher in rabbits supplemented with 100 and 200 mg vitamin C in feed than in water the reverse being the case at 300 and 400 mg vitamin C in the morning while in the afternoon skin temperature was higher in rabbits supplemented in feed at 100, 300 and 400 mg vitamin C than in water.




Morning Afternoon

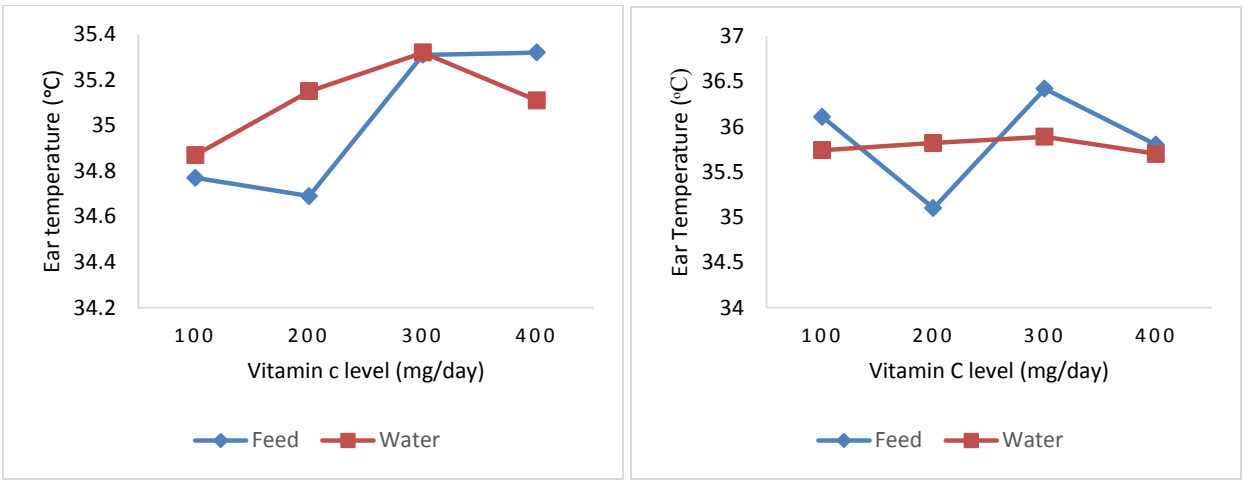
**Figure 2:** Respiratory rates of growing rabbits supplemented with vitamin C in feed and water in a humid tropical environment.




Morning Afternoon

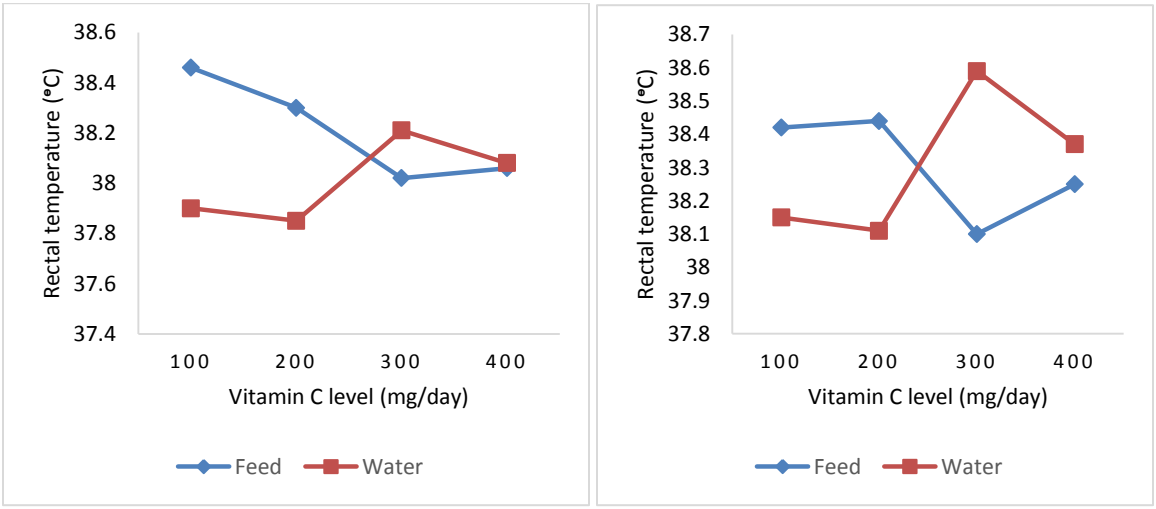
**Figure 3:** Skin temperature of growing rabbits supplemented with vitamin C in feed and water in a humid tropical environment.

Rabbits supplemented with 100 and 200 mg vitamin C had higher ear temperature in the morning (Figure 4), while in the afternoon, rabbits on 100 and 300 mg vitamin C in feed had higher ear temperature than those supplemented in water. Afternoon rectal temperature showed significant ($p<0.05$) interaction between vitamin C level and method of supplementation. Rectal temperature was lower in rabbits supplemented with 100 and 200 mg vitamin C in water in the morning and 100, 200 (water) and 300 mg vitamin C (feed) in the afternoon (Figure 5).




Morning Afternoon

**Figure 4:** Ear temperature of growing rabbits supplemented with vitamin C in feed and water in a humid tropical environment.




Morning Afternoon

**Figure 5:** Rectal temperature of growing rabbits supplemented with vitamin C in feed and water in a humid tropical environment.

## DISCUSSION

The rabbits thermo-neutral zone (TNZ) range between 18 and 21°C (Marai and Habeeb, 1994; Habeeb *et al.*, 1998). The ambient temperature during the study however, ranged between 22-23°C in the morning which is 2-3°C higher than the TNZ and 27-29°C in the afternoon, which is 4-8°C higher than the TNZ. Reduction in feed intake of rabbits as vitamin C level increased above 200 mg in this study could indicate a likely effect of acidity of vitamin C. Contrary to this, Afify and Makled (1995) reported increased feed intake of rabbits with increase in vitamin C inclusion in the diet. Bisong (2015) also reported significant increase in feed intake at inclusion level of 400 mg vitamin C.

However, Tuleun *et al.* (2011) and Dauda *et al*. (2015) did not observe significant differences in feed intake of quails or rabbits fed vitamin C.

Animals tend to consume more water during heat stress in order to replace or recover the water lost (King *et al.,* 1983) from the body. High water intake by rabbits on 200 and 300 mg vitamin C and in water supplemented, than the feed supplemented rabbits, would probably indicate the fact that rabbits preferred the taste of vitamin C in water than plain water. Adenkola *et al.* (2010) showed that vitamin C enhanced rate of water intake of rabbits. Bisong (2015) recorded high water intake at 400 mg inclusion level. Contrary to this study however, quails (Tuleun *et al.,* 2011) and rabbits (Dauda *et al*., 2015) did not show significant effect of graded levels of vitamin C supplementation on water intake. High water consumption by rabbits in the hot dry season helps to increase heat loss through respiration, evaporation, and urination (Chiericato *et al.,* 1992; Marai and Habeeb, 1994).

Significant effect of vitamin C on body weight observed in this study could be because of cumulative weight gain resulting from nominal differences in daily weight gains. This is more so, when considering the higher body weight of rabbits on 200 and 300 mg vitamin C supplementation in water than feed, with a concomitant higher daily body weight. It was expected that higher vitamin C supplementation would adequately ameliorate heat stress effects of the environment by increasing weight gain, an effect not observed with 400 mg vitamin C supplementation. This indicates a likely need for higher levels of vitamin C to adequately ameliorate the effect of heat stress resulting from the environment on growth of rabbits. Heat stress destroys cellular biological molecules (Liu *et al.,* 2011) and results in drastic damage to cell structure, protein, lipid, and DNA; and further induce physiological and pathological changes, resulting in poor performance (Abdel–Kalek, 2010). Apart from the reported changes in cellular integrity, daily body weight gain of rabbits was reduced in the hot dry season due to the negative effect of heat stress on the appetite centre with a resultant decrease in feed consumption rate (Marai *et al.,* 2002). Exposure to high ambient temperature and high relative humidity induced stress in rabbits, as observed in this study, though the rabbits were raised in temperatures ranging between 22-23°C in the morning and 26-30°C in the afternoon, but under high relative humidity (78-88%). Rabbits exposed to elevated ambient temperatures experience induced imbalances in their body temperature (Marai *et al.,* 1996, Habeeb *et al*., 1999), which adversely affect their growth (Habeeb *et al.,* 1996; Marai *et al.,* 2001, 2002; Okab and El-Banna, 2003; Okab *et al*., 2008) an effect that could explain lower body gains of rabbits in this study. Weight gains observed in this study are lower than 21.28-26.79 g/day reported for Hyla rabbits on vitamin C supplementation reported by Dauda *et al.* (2015).

Feed utilization by rabbits on vitamin C supplementation was high (1.61-2.53) indicating a high conversion of feed to weight gain and a possible improvement of feed utilization due to the possible effect of vitamin C supplementation. Adenkola (2010) and Dauda *et al*. (2015) reported similar improvement in feed utilization of piglets and rabbits on vitamin C.

Higher heart rate of rabbits in the morning in feed than water supplemented rabbits would indicate that supplementation of vitamin C in water aided in some level of amelioration of heat stress especially at 200 and 400 mg vitamin C supplementation however, in the afternoon, heat stress amelioration appeared better in feed than water supplementation at all levels of vitamin C supplementation. Heart rate increases when an animal is exposed to hot environment (Joshi *et al.,* 1982). The heart rate is one of the physiological response that give an immediate indication to climatic stress and consequently, the level of comfort or discomfort (Leagates *et al.,* 1991) and adaptability to an adverse environmental condition (Sethi *et al.,* 1994).

Heart rate of 180-260 bpm obtained in this study is within the range of 150-300 bpm reported to be normal for rabbits (Robertshaw, 2004). This is higher than 118.90-132.14 bpm (Dauda, 2014) and 140.99-143.53 bpm (Anoh, 2016) obtained for rabbits in a sub-humid tropical environment. Environmental temperatures have a significant relationship to variation in the heart rate, which also influences metabolic rate in animals (Ganaie *et al.,* 2013). Significant increase in heart rate in the afternoon compared to the morning on all the vitamin C levels indicates that the rabbits were under heat stress resulting from a likely inability of the levels of vitamin C to ameliorate heat stress in the afternoon. Increase in environmental temperature result in a corresponding increase in heart rate (Ganaie *et al.,* 2013). A negative correlation exists between air temperature and heart rate in animals (Chikamune and Shimizu, 1983). The increase in heart rate increases blood flow from the core to the surface to encourage heat loss by conduction, convection and radiation and through diffusion of water through the skin (Dauda, 2014).

The respiratory rates of rabbits observed in this study were high for all vitamin C levels and both supplementation methods. Respiratory rate increases when ambient temperature increase indicating a positive relationship between respiratory rate and environmental temperature which arises as a result of direct heat stimulation of the peripheral receptors, which transmit nervous impulses to the thermal cardio-respiratory centre in the hypothalamus, which send impulses to the diaphragm and intercostal muscles for further respiratory activity (Ganaie *et al.,* 2013). It is likely that the vitamin C levels used in this study are below the required quantity to effectively ameliorate heat stress in rabbits. Abdel-Monem *et al. (*2012) reported a significant reduction in respiratory rate of rabbits fed 800 mg/kg vitamin C over those without vitamin C inclusion in feed. High respiratory rate in most cases does not show that the animal is successfully keeping its body temperature constant, but rather indicates that the animal is over heated and trying to restore normal heat balance (Ganaie *et al.,* 2013). Though heart rate of the rabbits was within the normal range for rabbits, respiratory rates were far higher (125.55-142.69 cpm) than the normal rates (30-60 cpm) indicating that the rabbits were under heat stress even in the morning and especially in the afternoon. Ojebiyi *et al.* (2013 personal communication) reported pulse rate of 62.16-63.93 bpm for rabbits raised at ambient temperatures of 24.72-27.46°C and relative humidity of 62.02-77.46% in the humid tropics. While Ibiwoye *et al*. (2015) reported respiratory rates of 100-180 beats/minutes in Hyla rabbits supplemented with 100-400 mg vitamin C between the months of January to April in a sub-humid environment. A rise in respiratory heat loss through panting is one of the first physical signs of an animal experiencing heat stress (Nienaber and Hahn, 2007).

Higher ear temperature of rabbits on 300 and 400 mg than 100 and 200 mg would imply that rectal temperature and respiratory rate would be lower in the former rabbits than the latter. This was not observed in this study. Ear temperature was not affected by method of supplementation though it was higher in the afternoon than in the morning indicating an effect of increased ambient temperature in the afternoon. During the hot dry season, the animal stretches the ear pinna far from the body to dissipate heat through radiation, convection and evaporation to the surrounding, as a result of this processes, the ear temperature increases (Fayez *et al*., 1994). Skin temperature was not affected by vitamin C level nor method of supplementation though it was higher in the afternoon than in the morning, indicating an effect of high ambient temperature. High ambient temperature increases skin temperature of rabbits (Zeferino *et al.,* 2011), the skin serves as a receptor for cool or hot temperature where nerve impulses are being transmitted to the anterior and posterior hypothalamus for heat regulation and conservation, as well as feed consumption and

metabolic rate, which is influence by degree of ambient temperature (Willmer et al., 2000). Vitamin C significantly decreased skin temperature of rabbits during hot dry season (Adenkola *et al.,* 2009).

Significant increase in rectal temperature in the afternoon on all the vitamin C levels indicates an inability of the levels of vitamin C to ameliorate heat stress compared to the morning. In the afternoon rectal temperature indicated that at inclusion level of 100 mg and 200 mg, rabbits fed vitamin C in water had lower afternoon rectal temperature than those fed vitamin C in feed. More so at inclusion levels of 300 mg and 400 mg, those fed vitamin C in feed had lower afternoon rectal temperatures than those fed vitamin C in water. Rabbits fed 100 mg vitamin C in feed had the lowest rectal temperature. Rectal temperature rises as a result of high relative humidity, which reduces the efficiency of evaporative cooling coupled with high environmental temperature, to overwhelm the capacity of the animal to maintained normal body temperature (Ganaie *et al.,* 2003). Bisong (2015) recorded a significant increase in the rectal temperature with vitamin C supplementation which was attributed to low level of vitamin C indicating that at 100 mg inclusion level the rabbits could not ameliorate rectal temperature. High rectal temperature in New Zealand rabbit does and bucks as well as California bucks was also reported by Iyeghe-Erakpotobor *et al.* (2011) during the hot season than the cool season. Adenkola and Ayo (2009) demonstrated that vitamin C reduced the rectal temperature of animals especially during the hot hours of the day. This effect was not observed in this study in the hot afternoons. Similarly, Whitehead and Keller (2003) reported that, vitamin C ameliorates the adverse effect of heat stress by modulating and reducing the rectal temperature in a stressed animal. Ayo *et al.* (2011) reported a distinct diurnal fluctuation in rectal temperature of rabbits. Balabel-Mousa (2004) observed that rectal temperature in rabbit groups treated with vitamin C was lower than the untreated group. Rectal temperature obtained in this study is within the range of 37-40°C reported for rabbits (Robertshaw, 2004).

Considering the ambient temperature of the environment which is close to the recommended temperature of the thermoneutral zone, especially in the morning, it would be expected, that the thermoregulatory parameters of the rabbits such as heart rate, respiratory rate and rectal temperature, should be within the normal range or only slightly higher than the normal rates especially in the morning. This was however, not the case with respiratory rates of the rabbits which were far higher than the normal rate. Based on the high respiratory rates and heart rates and moderate daily weight gains and low feed intake of the rabbits, despite supplementation with vitamin C in feed and water, it is concluded that 200 mg vitamin C in water and 300 mg vitamin C in feed is recommended to ease heat stress effect in this environment. The levels of vitamin C used in this study was not sufficient to elicit an optimum amelioration of the evidently heat stressed rabbits therefore higher levels should be evaluated.

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